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Inhibition of *C. difficile* and *C. perfringens* by commercial and potential probiotic strains and their in-vitro growth characteristics

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Probiotics have gained importance in human and veterinary medicine to prevent and treat clostridial associated enteric disease. Little information is available on commercially produced potential probiotic bacterial strains regarding their inhibition of *C. difficile* and C. *perfringens* and their growth characteristics. The objective of this study was to determine the inhibitory effect of commercial and potential probiotic on *C. difficile* and *C. perfringens* and assess their growth characteristics in-vitro.

The inhibitory effect of a cell free probiotic supernatant of 17 commercial bacterial strains (Lactobacilli n=16, Bifidobacteria n=1) on growth of clostridia spp was assessed in an agar well diffusion assay and broth co-culture experiment, using supernatant harvested at different growth phases and with and without pH adjustment. To study growth characteristics MRS broth was adjusted to pH2 or pH4 or supplemented with 0.15% or 0.3% bile and growth was compared spectrophotometrically between standard and modified broths. In the agar well diffusion assay 2/17 probiotic strains inhibited *C. perfringens* independent of the pH of the supernatant and independent of the growth phase it was harvested. 10/17 probiotic supernatants inhibited *C. difficile* in a pH dependant manner when harvested in the stationary growth phase. In the broth co-culture 5/17 probiotics inhibited *C. perfringens* and 10/17 inhibited *C. difficile* both in a pH dependant manner. All probiotic strains were able to grow at pH4 (growth range 44-90%) but none were able to grow at or survive pH2. All probiotics were able to grow in 0.3% and 0.15% of bile, growth ranged between 44-91% and 56-99% respectively.

5/17 probiotics tested showed good inhibitory potentials against both *C. difficile* and *C. perfringens* and also showed favourable *in-vitro* characteristics for use as potential probiotics and should be further studied in in-vitro studies and double blinded randomized placebo controlled clinical trials for prevention or treatment of *C. difficile* and *C. perfringens* associated disease.